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# Probiotics as a Promising Additive in Broiler Feed: Advances and Limitations

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## Abstract

Feed additives have a strong influence on the production cost of broilers as growth-promoter's to cover variations in profits due to fluctuation in feed costs. Antibiotics as additives were fundamental and indispensable, however, studies have shown their connection with the emergence of resistant strains of pathogens in animals and humans, therefore in recent years they have been less encouraged. Research is in progress concerning additives that can replace antibiotics as growth promoters and also as prophylactics. It was demonstrated that probiotics, which are living microorganisms and without residual effect's have a potential to be used as microbials. However, they are not always guaranteed as growth promoters, as there are mechanisms of action regarding their interaction with the host that cannot yet be properly understood. The main advances in the use of probiotics in broilers in recent years, as well as the gaps, challenges, and future perspectives were carefully discussed and analyzed in this study. It was considered as a future premise, the possibility of reviewing the traditional methodologies used to test the hypotheses related to the effect of probiotics in broilers, which may also be extended to other animal species.

**Keywords:** feed additives, intestinal health, use of nutrients, antibiotics, performance

## 1. Introduction

Broiler chicken is an affordable and complete source of protein for human consumption. In addition, it is a source of income for families, small and large companies around the world [1, 2]. In broiler breeding, intestinal health is crucial, due to its influence on health and use of nutrients [3]. Furthermore, its influence directly in obtention of the ideal performance parameters. Special attention must be paid to the gastrointestinal tract, as it is where most disease-causing pathogens enter, settle and multiply [4]. In the same way preventing or treating diseases of the tract results in more efficiency for other bird body systems [5].

After hatching, the intestinal tract of birds is sterile, which becomes populated with microorganisms when searching for food, constituting their intestinal flora [3]. Intensive breeding coupled with confinement applied in modern production of broilers limits the movement of birds putting them at risk of health vulnerability and dependence on the provided diet [6].

Various additives with different functions have been used to formulate complete diets for broilers. Some additives act as nutrition increments' and others guarantee protection against disease. Antibiotics were among the most used additives to control the birds health [7].

Antibiotics were widely used as growth promoters, whose application was made in sub-therapeutic doses throughout the breeding of broilers. However, strong evidence indicated that antibiotics contributed to the development of resistance in pathogenic strains [8, 9].

The emergence of resistant strains in poultry farms have been rousing a major concern. Reports have proven not only the damage on the animal's health but also to human due to the trophic interactions [9, 10]. Consequently, there are major concern to withdraw antibiotics from broiler chickens. The search of alternative sources of additives that would be potential substitutes for antibiotics in broiler chickens become a crucial issue for many researchers [11]. Nonetheless, the use of antibiotics presents economical concerns, because beside to aforementioned advantages they acted in the control of diseases and in reducing the burden of carcass contaminants [12].

Probiotics became a potential antibiotic substitute additive in broilers, due to its influence in performance of broilers and security compared to antibiotics [13]. Probiotics are groups of non-pathogenic microorganisms, normally inhabitants of the intestinal flora, which, when administered have the benefit of balancing the intestinal microbiota by inhibiting the excessive multiplication of pathogenic microorganisms [3]. In addition, the presence of these in the tract activates the host's immune system, stimulating them to remain alert against any invasion [14].

Probiotics had already been applied to humans and other animal species. Certain species of microorganisms among which bacteria, fungi, and yeasts are carefully selected to be used as probiotics [15].

In broiler, however, even today, it is still not possible to accurately predict the effect of probiotics, due to several intrinsic and extrinsic factors that interfere with their use and results tend to be different between studies. The purpose of this study is to critically analyze the main findings regarding to probiotics applications in broilers.

## **2. Probiotics in broilers, concept, types and effect**

Probiotics in animals as well as in humans are being extensively studied, due to its effect on the restoration of the intestinal microbiota, reversal of dysbiosis (gastrointestinal imbalance), and safety in its use [16, 17]. The use of antibiotics is banned from the European Union (EU) and other parts of the world, catalyzed the research of probiotics for broilers. In addition, the antibiotics ban, not only affected countries importing meat but also pressured the meat exporting countries to meet the regulations of receipt countries [10, 18].

The use of antibiotics in animals is prohibited in the EU and the USA, is the practice responsible for creating antibiotic-resistant bacteria, known as superbugs. These superbugs having animals as reservoirs can spread among other animals, management personnel, food and the environment [12].

Some portfolios have been developed possible alternatives to antibiotics [11], these have included probiotics in the most advanced approaches to serve as preventive, and alternative therapies with potential uses in animal health. Derived from Greek, the word probiotic means "pro-life" and in the search to cover the correct mechanism of probiotics, this term has undergone several changes over the years. In animal production, it can be summed up as a "feed supplement for live microorganisms that beneficially affects animal flora by improving the microbial balance in the intestine" and should be viable and stable in the different breeding conditions [19].

The most used microorganisms as feed supplements are mainly bacteria, mostly from the group of Gram-positive *Bifidobacterium* and lactic acid bacteria (LAB). The genus *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Streptococcus*, *Lactococcus*, and *Leuconostocos*, and also *Bacillus* are the most used. In addition to bacteria, fungi and yeast strains, mainly from the species of *Saccharomyces cerevisiae* and *kluyvero-mycetes* are also used as probiotics [8, 19, 20]. A microorganism to be considered as a probiotic must meet some requirements: (1) to resist and quickly occupy the intestinal tract; (2) be part of the intestinal habitat; (3) survive the action of diges-tive enzymes; show antagonistic action against pathogenic microorganisms; (4) be non-toxic and non-pathogenic; (5) be stable and viable in commercial preparation and stimulate the immune system [21].

Several probiotic strains are included in poultry diets to promote animal growth and health, especially when conditions are challenging for health. Several studies have reported the beneficial effects of probiotics on various aspects of bird health. Furthermore, parameters related to supplementation with probiotics in diets increased body weight gain, feed intake and improved feed conversion rate in birds, were also reported (**Table 1**).

Until now, many mechanisms about the action were proposed. However, the main mechanisms of action of the probiotics are competition for binding sites, where the probiotics adhere to the intestinal epithelium wall, hindering competi-tion and the joining of pathogenic microorganisms, this higher concentration of the beneficial microbiota also causes it to have advantage in competition for nutrients [12]. Supplementation with *Bacillus subtilis* improves the performance and immu-nity of broilers reared in warm conditions, and birds fed probiotic in the diet were able to deal more effectively with heat stress through immunity modulated by the

Parameters evaluated	Probiotic strains used	Source/Author
Promotion of feeding efficiency and improvement of subclinical necrotic enteritis	<i>Bacillus amiloliquofaciences</i> H <sub>57</sub>	[22]
Intestinal barrier, antioxidant capacity, apoptosis and immune response	<i>Lactobacillus plantarum</i> 16 and <i>Paenibacillus polymyxa</i> 10	[23]
Performance in broilers	<i>Bacillus coagulans</i> and <i>Lactobacillus</i> sp.	[24]
Use of nutrients	<i>Buttiauxella</i> sp. and <i>Bacillus</i> sp.	[25]
Performance, apparent ileal digestibility, blood and excreta characteristics	<i>Lactobacillus acidophilus</i> ; <i>Bacillus subtilis</i> and <i>Clostridium butyricum</i>	[26]
Improving the stress of stocking density, yield	<i>Lactobacillus acidophilus</i> , <i>L. casei</i> ; <i>Enterococos</i> and <i>Bifidobacterium termophilus</i>	[27]
Immune performance and protection	<i>Lactobacillus acidophilus</i> , <i>L. casei</i> , <i>Enterococos faecium</i> and <i>Bifidobacterium bifidum</i>	[28]
Energy digestibility, performance, disappearance of non-starch polysaccharides	Multiceps of <i>Bacillus</i>	[29]
Hatching of fertile eggs	<i>Lactobacillus acidophilus</i> ; <i>Bacillus subtilis</i> and <i>Bifidobacterium animalis</i>	[30]
Microbial profile of cecum and litter	<i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> and <i>Bacillus megaterium</i> (spores)	[31]
Resistance to heat stress	<i>Bacillus subtilis</i>	[32]

**Table 1.**  
Most evaluated parameters in broiler probiotics uses.



microbiota [33]. Other studies, showed that nutritional effects where probiotics act by increasing fiber digestion in birds and enzymatic activity [34]. A competitive inhibiting effect, in which high amounts of *Lactobacillus* bacteria's produce organic acids that enable low pH in the crop that suppresses the colonization of pathogens in the digestive tract were also observed [21].

Testing different strains (*B. subtilis* DSM 32324, *B. subtilis* DSM 32325, and *B. amyloliquefaciens* DSM 25840) in isolation and in combination were showed that the tested strains had different abilities to degrade proteins and carbohydrates and inhibit the growth of *C. perfringens* in vitro. The in vivo results demonstrated that combined strains can act more efficiently than isolated strains on performance parameters as well as reducing mortality in birds challenged by *C. perfringens* [21].

Another study [35] used combined strains of *Bacillus licheniformis* and *Bacillus subtilis*, in order to investigate their effects and found that probiotic supplementation can increase profits if associated with dietary restriction. In addition to the feed efficiency, there was no influence on the carcass yield, the relative weights of the liver, gizzards, proventriculus, small intestine and bursa of fabricius, and the visible fat of the carcass was reduced. The association of probiotics with prebiotics was strongly recommended by these authors.

There is a belief that multiple strains combined with prebiotics have a better effect than isolated strains. FAO reported [20] on the use of probiotics in animals states that the benefit of using more than one strain in the same product still not clearly established.

### 3. Challenge of replacing antibiotics with probiotics

The recommendation for antibiotics to be replaced by other compounds has been the subject of discussions around the world, mainly after the European Community officially banned its total use in animal nutrition, with the disclosure of Regulation (EC) no. 1831/2003. The main reason for substitution is the occurrence of cross-resistance to drugs used to treat bacterial infections in humans [10]. According to the joint report by the World Health Organization (WHO), Food and Agriculture Organization (FAO) and World Animal Health Organization (OIE), there are still countries that do not yet have control over antibiotics that circulate in animal production (Africa and the Latin America). Many of them lack an organizational structure for control and others only limited their use to promote growth of the animals. Most of the European countries have adopted strict measures to control antibiotics that are circulating throughout the food chain and used in hospitals [9].

Following the recommendations of world health agencies, meat exporting countries, such as Brazil, for example, as the largest meat exporter in the world have been adapting to the demands of international markets. Through the prohibition of antimicrobials commonly used as growth promoters in animal production. Through the normative instructions (IN), the Brazilian Ministry of Agriculture and Livestock has implemented the ban on the following substances as a performance-enhancing zootechnical additive: Olaquinox (IN n°. 11, November 24, 2004), carbadox (IN n°. 35, November 14, 2005), spiramycin and erythromycin (IN n°. 14, May 17, 2012), colistin sulfate (IN n°. 45, November 22, 2016) [36], and the substances bacitracin, tylosin, lincomycin, virginiamycin and tylosin (IN n°. 171, December 13, 2018), [37]. The inhibition is mainly for the purpose of export according to the recipient's rules.

To overcome a challenge of antibiotics concerns for human and animal health, substantial research that investigated alternative increased exponentially. Compounds, such as herbal medicines, [38, 39] prebiotics [40], organic acids [41],

symbiotics and probiotics [42] aiming to achieve results like that of antibiotics has been carried out. Economic and environmental impacts may occur with the ban of antibiotics according to [43], implying an increase in production costs of edible noble meat, increase in water consumption and in the production of excreta, as it was observed higher feed consumption and worsening feed conversion when no antibiotics are used. However, the impacts with the frequent use of antibiotics resulting in the selection of resistant strains can be more drastic [44].

No significant differences was observed for the performance characteristics in the period from 1 to 21 days in birds of the group of the zinc bacitracin antibiotic, when compared to the group of a probiotic composed of *Lactobacillus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, and *E. faecium*, with better weight gain being observed for these two groups in relation to the group without the addition of additives [45].

Studies carried out with the strains *Lactobacillus* spp., *Bifidobacterium bifidum*, *Streptococcus thermophilus* and *Enterococcus faecium* indicate the efficiency of probiotics against pathogenic microorganisms when compared to conventional antibiotics, being able to reduce colonization by *Salmonella enteritidis* in the intestinal segments [46], without changes in feed consumption and feed conversion [47]. The practices of biosecurity in production combined with the use of probiotics are the way to maintain production efficiency and quality of the final product in the current scenario, [17, 48].

Supplementation with *Bacillus subtilis* probiotic strain reduces infectious agents, such as *Salmonella*, improving the intestinal digestive and absorptive efficiency, in which the probiotic is able to control the population and load of cecal *Salmonella*, being similar to the effect of the antibiotic enrofloxacin [49]. According to WHO (2016), salmonellosis is considered a disease that affects people worldwide, and that has some serotypes resistant to conventional antimicrobials [50].

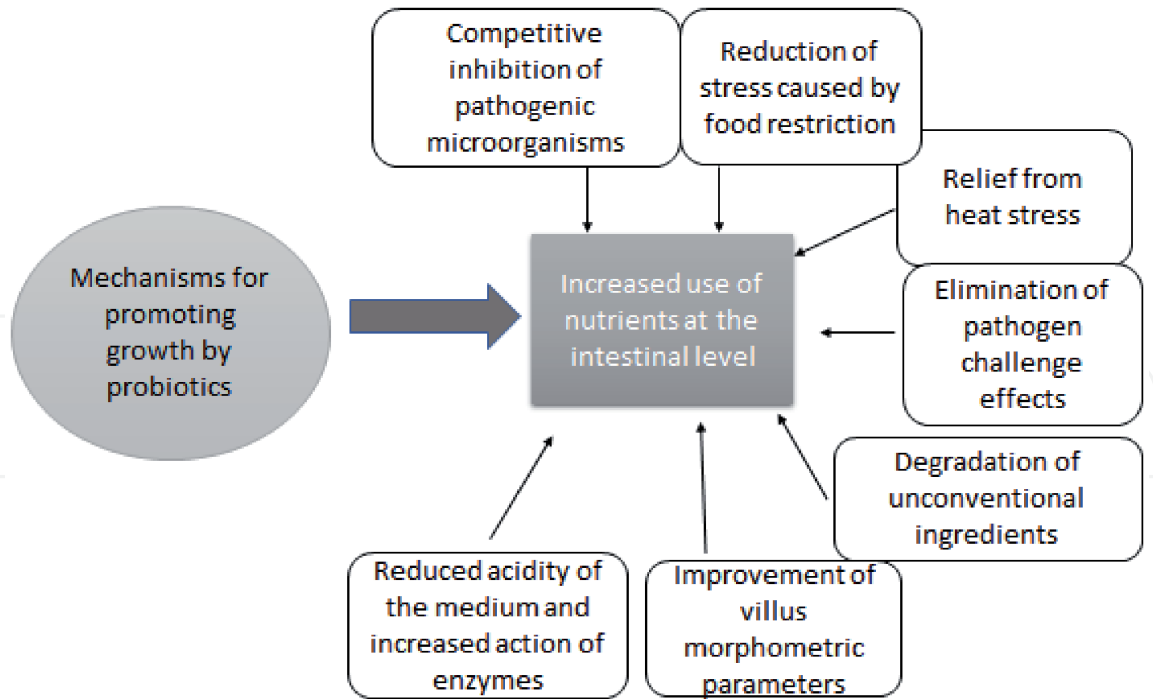
Corroborating the research carried out by [51] in Brazil, that when assessing the prevalence of several *Salmonella* spp., serotypes in chicken carcasses and live chickens in slaughterhouses, found that 50% of establishments had a prevalence above that recommended by Ministry of Agriculture, Livestock and Supply (MAPA). The same authors evaluated the resistance of *Salmonella* spp. serotypes to two groups of antimicrobials, fluoroquinolones and beta-lactams, and observed that the strains were more resistant to beta-lactam antimicrobials, which demonstrates the risk in the treatment of clinical conditions of salmonellosis in humans.

Another study [52] showed that the use of probiotics, and probiotic combined with 1% garlic powder, was able to promote villi with good width and height in the small intestine and improvements in the performance of birds in the absence of antibiotics, although no improvement in feed intake has been observed. The antimicrobial characteristics of garlic combined with the probiotic reduce the microbial load that in response optimizes the absorption of nutrients in the small intestine.

Although lower feed consumption is observed in some studies, the probiotic allows improvements in other characteristics of greater economic importance such as body weight and feed conversion [53]. Lower feed consumption can be influenced by factors such as heat stress and environments with greater health challenges [54].

#### 4. Promoting growth mechanism by probiotics in broiler

Naturally, the beneficial microbiota that inhabits the intestines of broilers uses a competitive suppression mechanism (as shown in below **Figure 1**) to reduce the multiplication of others that are pathogenic, preventing damage to the mucosa by



**Figure 1.**  
*Possible mechanisms of performance promotion by probiotics.*

irritating toxins, controlling enteritis, and guaranteeing protection to the mucosa [5]. Mechanisms are described according to which microorganisms (1) competes for the site of adherence in the mucosa, for better use of nutrients and greater reproduction, in which the one in greater quantity will serve as a barrier that restricts the adherence of others in lesser quantity, reducing possibilities of the latter to multiply; (2) there is the production of organic acids that lead to a reduction in the pH of the medium and the establishment of an acidity that eliminate or decreases all intolerant microbiota, the majority of which are pathogenic; and (3) there is an activation of the immune response by the receptors (toll-like) present in the wall of beneficial bacteria, which, when in contact with the cells of the intestinal mucosa stimulate them to produce immunoglobulins, that will inhibit the multiplication of the pathogenic microbiota [18].

In environments where chickens are subjected to heat stress, probiotics in association with trace minerals, can help maintain or even improve performance parameters, resulting from the increase in the surface area of the intestinal villi [55].

The authors [56] found that the use of probiotics improves blood biochemical parameters, reduced serum uric acid concentration, modified intestinal microstructures and reduced enterobacteria in the ileum and cecum.

Probiotics in combination with other natural additives helps to make better use of the nutrients of alternative ingredients, which for the most part, have anti-nutritional factors, enabling the best use of these for the production of animal feed [57]. [58] concluded that broilers fed 15% of shea butter cake flour added to probiotics, obtained better carcass characteristics and noble cuts when compared to the control diet without probiotics and without flour.

Probiotics act in the restoration of intestinal microflora, decreasing inflammatory processes caused by pathogenic bacteria, with an increase in villus height, and improving zootechnical performance characteristics [59]. This effect was achieved due to competitive exclusion, reduction of enterotoxins and direct antagonism [60]. Other work also show that the use of probiotic improves the body weight and feed conversion of birds, when observing the increase in the levels of glucose and

albumin in the blood, which indicates better digestion and absorption of nutrients. The same authors suggest that studies must be carried out to investigate the ideal concentration of probiotic in the feed [61].

The modes of action of probiotics are generally not understood. When summarizing the advantages of using probiotics, one should emphasize their role in protecting animals against pathogens, increasing the immune response, reducing the need for antibiotic-based growth stimulants and high safety of these formulas [19], these factors all contribute to the greater use of nutrients by the bird and, consequently, to a better performance.

## 5. Limitations on the use of probiotics in broilers

The limitations surrounding the application of probiotics are several, however those inherent in the breeding environment are those described here, among which are summarized below.

### 5.1 Absence of a pattern in the effect of probiotics

The absence of a common pattern in the effect of probiotics and their probiotic potential is directly connected to particular species, not to the genus or species of a microorganism. Studies with *Bacillus subtilis*, analyzing from performance to biochemistry parameters showed variable results [21, 24, 62–64].

Using Probiotics based on *Bacillus subtilis*, bone growth in broilers under an episode of cyclic heating was induced, which was attributed to the inhibition of bone resorption, resulting from the negative regulation of circulating TNF- $\alpha$  and CTX, [32, 65].

In chickens challenged with *C. perfringens* tested strains of *Bacillus subtilis*, they showed no significant difference in the ability to degrade proteins and carbohydrates and inhibit the growth of *C. perfringens* in vitro, when compared to the control without probiotics, [21]. Already combined with *L. amiloquaficiences*, the results demonstrate the suitability of the combination of multi - strains of *Bacillus* evaluated as an effective probiotic.

A study by Liang [64], showed that probiotics (*Bacillus subtilis* and *Lactobacillus acidophilus*) in isolation, were not effective in controlling *E. coli* infection in broilers and did not lead to significant weight gain. In addition, 75% of the mortality was obtained in treatments with the application of these probiotics. The combination of these probiotics with flavonoids (*Taraxacum*) improved indicators of diarrhea [64].

According to assessments made on the use of probiotics in animals by FAO the effects of probiotics seem to result from their interaction with the host. Such interaction is likely to define the mode of action of probiotics, which may be similar in different probiotics, or a specific strain may function through various mechanisms and several strains of probiotics have similar effects on the gastrointestinal microbial population [20].

### 5.2 Post-marketing handling and conservation

The stability of the probiotic in the storage time is one of the criteria of useful technology established to demand from formulators [66]. However, situations of inadequate handling or conservation after acquisition are more likely to happen and may result in inactivating their effect, and consequent absence the effect now expected, since it is a live microorganism.



The probiotic formulas, depending on the species and age of the host animals, can be administered as a powder, suspension, capsules, pellets, gel or paste [67]. In broiler chickens the most usual form of administration depends on the manufacturer, usually applied via injection (in eggs); via litter; via suspension and also orally.

In oral application conventionally made by means of food or water, the probiotic may be subject to loss of viability in contact with water disinfectants (chlorine) or by interaction with other substances contained in feed. Probiotics are not always effective in reducing microbial load in the same proportion, their effectiveness under commercial conditions is not guaranteed [68].

Eggs injected with probiotics before incubation to verify the effect on their hatchability, had unsatisfactory results, in which no protective effect was obtained [30].

### 5.3 Existence of internal animal or environmental challenges

Several factors can induce stress in chickens, which results in a reduction of beneficial microorganisms throughout the intestinal tract and their inefficiency in their protective action. The same analogy is used in the administration of the probiotic, in minimum concentration values it is necessary to ensure that they are available until reaching the site of action in the intestinal tract.

With the chicken's intestinal microbiota variable, due to factors such as geographic location, breed, temperature and dietary ingredients, it is very possible that other commensal bacteria can nullify, reduce or amplify the effects that probiotics can have on animal behavior and neurochemical metabolism [69].

The use of combined probiotic (*L. plantarum*, *Lactobacillus rhamnosus*, *Enterococcus faecium*, *Candida pintolepesii*, *Bifidobacterium bifidum* and *A. oryzae*) had no statistical difference when compared to the control without probiotic, did not affect the performance, internal organs and blood parameters of the chickens challenged with *Clostridium perfringens* [34]. However, in preliminary in vitro studies, this same mixture of strains showed abilities to inhibit the growth of the pathogen.

The mechanism by which the effects of stress or challenge are reversed are associated with the stimulation of the immune system, which in turn activates the nervous system and occurs the restoration of body balance and the fight against the pathogen [46].

### 5.4 Lack of suitability of the probiotic manufacturer

There seems to be no rule for combining probiotic strains in a formulation, nor an indication of the expected effect of such a mixture to treat a specific problem, which strengthens the use of probiotics as prophylactics rather than as therapeutic. [19, 20, 69, 70].

Information about the formulation is extremely important, especially for commercial broiler production farms or other animals that routinely use probiotics or other prophylactics (live, inactivated, and similar subunits) in their animals without knowing if there is an improving or canceling the effects of each other [69].

Some commercial formulas of probiotics combined, are still sold with indefinite constitution of the strains. A study was carried out with probiotic compounds with undefined strains that were purchased from authorized local stores. That, tested in the treatment of broilers challenged with *Salmonella enteritidis*, the positive effect

was only in the first 5 days [46]. There is an assumption that probiotic strains reduce the concentration over time throughout the intestine [70] and, for the best effect, continuous inoculation is necessary to maintain the optimal levels of the probiotic load, capable of competing favorably [18]. Even so, the possibility of dishonesty on the part of the manufacturer is not ruled out. According to the established standards FAO [9, 20] for the manufacture of probiotics, species and strains, as well as minimum viable quantities must be respected and declared.

Studies carried out due to the performance of tests to confirm the information contained in the label of probiotics of several commercial brands, it was found that there were numerous irregularities, highlighting whether (1) existence of strains different from those declared; (2) Encapsulation material not suitable for animal consumption or to maintain viability within the stated period; (3) association of species or strains other than those declared and (4) non-viable microorganisms in packaging within the time limit [71–75].

If there are inconsistencies in the results of probiotic research, information on the safety of a specific microorganism should not be applied to other closely related microorganisms.

## **6. Risks inherent in the use of probiotics**

The absence of clinical side effects is an important benefit of using probiotics. However, some of the species and/or bacterial strains can present risks, they can be responsible for a series of problems to animal, human health and the environment, causing mild reactions or serious and potentially fatal infections [20]. In addition, no probiotic can be considered 100% safe or at zero risk.

Risk reports on the use of probiotics are scarce. However there is a probable risk of transmission of antibiotic resistance (eg, *Enterococcus* genus) to pathogenic microbiota or enterotoxin production (eg, *Bacillus cereus* strain) [19].

In the USA, probiotics and other microorganisms used as food additives must have the status of Generally Regarded as Safe (GRAS), regulated by the FDA. In Europe, the use of the term Qualified Safety Assumption (QPS) has been regulated by EFSA. Crucial criteria for the selection of probiotic microorganisms in animals were jointly established by (WHO), (FAO) and EFSA, where they stand out: Security, functionality and technological utility [19].

Security - stipulates that the origin of the strain to be used as a probiotic must be from the intestine of this animal species; whose antibiotic resistance profile must be non-existent; in addition to the need for a total absence of association with pathogenic cultures.

Functionality- as an important criterion, functionality is about the viability of the strain within the conditions of the intestine, which must be greater; in addition to the efficiency in adherence to epithelial cells, reduced intestinal permeability of the mucosa and proven immunomodulatory effects.

Technological utility - no influence of probiotic microorganisms on the sensory properties of meat or meat products should be observed. Phage resistance is also an important property in this criterion, as well as the guarantee of viability of the microorganism during processing, and good stability in the product and in the storage time.

Current levels of information about probiotics are not sufficient to declare any group of probiotics completely safe without a case-by-case risk assessment. The results of the studies do not allow establishing the existence of a real risk for probiotics, but they also do not guarantee total safety.

## 7. Final considerations

There is still a long way to go and studies must be done to optimize probiotic formulas in order to guarantee the desired effect. Promising steps were evidenced with the administration combined with other strains of the same or different species and also with other additives such as: phenolic compounds, enzymes, prebiotics and unconventional ingredients.

Generally, the efficacy of probiotics in broilers is evaluated by parameters such as weight gain, feed conversion, and resistance to pathogens, ignoring other aspects such as commercial or natural conditions of the product used, its interaction with feed components and the environment, and their impact on the animal's response. During the use of probiotics, other mechanisms by which these live microbes can affect the host, such as the role in the maturation of the nervous and enteric systems that mediate animal behavior through the gut-brain-microbiota axis, need to be considered.

Even with validated effects of probiotics in several studies, research still shows that the different responses found suffer influences related to environmental factors, specific to the host or the strain, dosages recommended by the manufacturers of the products, types of strains used either alone or in combination, joint use of zootechnical additives, as well as different experimental conditions and greater or lesser health challenge in the broiler breeding phases.

Considering all of the aspects described above, new approaches to checking the effects of probiotics will be possible, and it would be interesting to go beyond the usual comparison that focuses on "treated animals compared to untreated animals" that is observed in most studies to assess effectiveness of probiotics. Both in research and in the application (judged by the supplier market) of probiotics, the role they play as an additive is evidenced, not exactly as a growth-promoting additive, but one that improves, restores, and or installs the optimal conditions for the obtaining the best performance in broilers.

## Conflict of interest

No conflict of interest

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